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1.—SARCOSPORIDIA.

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INTRODUCTION.

Sarcosporidia are, as their name implies, parasites of muscle. Their systematic position is uncertain. They are customarily placed among the Protozoa, Wenyon (24) regarding them as parasites of undetermined position.

The first discovery of Sarcosporidia was made in 1843 by Miescher. They were observed in mice and for some time were called Miescher's tubes. Rainey was the next observer, and he found the parasites in pigs in 1858, but regarded them as young forms of *Cysticercus cellulosae*. Leukhart corrected the mistake and showed that the parasites were closely related to the *Myxosporidia*.

Wenyon (24) commented on the fact that Ashworth showed the *Rhinosporidia* were fungi, and suggested that the *Sarcosporidia* may be of a similar nature.

Sarcosporidia are commonly found in voluntary muscle, but may occur in involuntary and heart muscle. The Sarcocyst elongates within the fibre at the expense of the muscle substances, which is destroyed in the process. In such a way a single parasite may grow to several millimetres in length. Occasionally the parasites become calcified *in situ*.

It is debatable whether the Sarcocyst found within the muscle fibres may be regarded as a single parasite; it is more usual to regard this period as a cyst stage, for a minute examination shows that the contents are divided up into sickle-shaped bodies which are regarded as spores.

McGowan (17) described the cuticle of the Sarcosporidial cyst as being covered on the outside with cilia which interpenetrate the muscle fibre substance. Immediately beneath the cuticle is a layer of cells which is supposed to be sporoblastic in nature, the component cells by successive divisions produce immature sickle-shaped spores which later ripen. The sickles near the centre of the cyst seem to lose their chromaticity and degenerate. The cuticle of the cyst is regarded by some authors as containing a number of fine pores, which are, however, too small to allow the sickles to escape. Under higher magnification McGowan showed the sickle possesses a well-defined structure. Almost one-half is occupied by a dark non-granulated zone, and the other half, which is full of granules, contains the nucleus.

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Balfour (1) found that the Sarcocyst of the Gazelle possessed two kinds of spores, which stained differently with Toluidin Blue.

Wenyon (24) states that Alexieff considered the Sarcocyst to be composed of a large number of uninucleate parasites, and that the membrane which encloses them and the septa which divide them up into chambers, have been derived from the host.

According to Fantham (7) the stage during which the parasite elongates within the muscle fibre is known as the Trophozoite stage, and the contents, the sickle-shaped spores, represent the Sporozoite stage.

The residual masses of protoplasm which are left when the sickles are formed, and which by successive divisions produce further sickles, are known as pansporoblasts.

McGowan (17) found that on degeneration of the sickle, the contained chromatic granules are set free, and he regards these as the infective agents of the Sarcocyst.

Braun and Lühe (2) stated that the spores of the *Sarcosporidia* of mice have been found to be motile, "progressing in a series of screwing jerks."

DEVELOPMENT.

Numbers of experiments have been carried out by various authors in order to ascertain the mode of development of the Sarcocyst. Mice have been fed with meat infected with *Sarcosporidia* and the presence of Sarcocysts in the voluntary muscles has been noted about forty days after the meal. The manner in which the Sarcocysts proceed from the alimentary canal to the voluntary muscles is not definitely known, but McGowan (17) suggested that the small chromatic particles in the sickle may be set free on degeneration of the Sarcocyst, and may travel through the tissues to the voluntary muscle fibres. He quotes the experiment of Van Betegh and Dorisch, who fed ducks on the *Sarcosporidia* of sheep and found early stages of the parasite in the musculature of the stomach, each stage being composed of three chromatin particles, associated with a mass of protoplasmic material. McGowan considers that if these small chromatic particles can wander into the stomach muscles, there is nothing to stop them wandering into a fœtus in utero. Stockman (20) and Gaiger (8) consider this possibility as being extremely unlikely.

Wenyon (24) considers that the rupturing of the spore membrane liberates a small amœbula type of body, which effects an entrance into the intestinal cells. These persist only for a few days during which they undergo a period of multiplication in the region of the gut and then disappear; reappearing sometime later, about forty days, in the muscle fibres.

Fantham (7) gives an account of the descriptions of Erdmann and Crawley on the development of the Sarcocyst.

According to Erdmann, the spore on ingestion by the host, germinates, thereby liberating its contained toxin, which by its action on the surrounding epithelial cells, enables the amœbula which forms from the spore, to enter the intestinal lymph spaces, and then proceeds, after resting for about a month in these spaces, to the muscles.

Crawley considers that the spore or sickle is able to penetrate the epithelial cells by boring, and that once inside, it loses its sickle shape and rounds up, and that it may in this stage undergo multiple fission, and that, in fact, its appearance suggests such an occurrence. This worker believes that the spores are sexually differentiated.

The young forms of the Sarcocyst appearing in the muscle fibres at the end of forty days, are small and contain very few nuclei. These increase in number and then, according to Wenyon (24), the mass of protoplasm divides itself off around each nucleus, and a number of spores are formed equal in number of the nuclei.

When the Sarcocyst has finally destroyed the muscle fibre in which it lies, it breaks the sarcolemma and its own membrane and comes to lie free in the connective tissue of the muscle, when the spores proceed to infect other muscle fibres.

OCCURRENCE IN HOST.

The *Sarcosporidia* in Mammals, occupy chiefly the muscles of the œsophagus and larynx, and the diaphragm and body wall. In acute infections any of the skeleton muscles may be infected, even the eye, tongue, and cardiac muscles.

PATHOGENICITY.

Sheep commonly show the presence of Sarcocysts in their voluntary and involuntary muscle fibres, but these do not appear to cause any noticeable ill effects. McGowan held the theory that a particularly heavy infection may cause a sheep disease which is known as "Scrapie" in England, "Traberkrankheit" in Germany, and "La Tremblante" in France. Gaiger (8), however, has come to the conclusion that *Sarcosporidia* have no connection with this disease.

One of the commonest of Sarcosporidial infections in sheep is that caused by the genus known as *Balbiana*, which causes tumours in the œsophagus of the infected animals. *Balbiana* has been found in Western Australian, New South Wales, Victorian, and New Zealand sheep. The form known as *Balbiana gigantea*, is believed to be the same as *Sarcocystis tenella* (Raill) which is frequently found in the skeletal muscles of sheep.

Since *Sarcosporidia* are common in sheep and they are known to secrete a very virulent toxin it is possible that even a very small infection may have a deleterious effect on the good health of the infected animal, perhaps weakening its resistance and making it more liable to other infections, or dietary deficiency. No symptoms resulting from Sarcosporidial infection have been observed in Western Australian sheep.

The Sarcocyst infecting mice is very deadly. The author observed that a mouse infected with *Sarcosporidia* assumed a semi-waddling gait, as if its hind legs had been broken, that it became very swollen and puffed up, and eventually died.

Little is known of the effect of other *Sarcosporidia* on the animals infected. In one of the cases of human infection recorded by Wenyon (24) the subject complained of pain in the muscles concerned. It is possible that Sarcosporidiosis may occur more often than is suspected in human subjects.

TRANSMISSION OF SARCOSPORIDIA.

One can easily understand how Sarcosporidiosis is spread in carnivorous animals, but the specificity of the infection is not known. Attempts have been made by various workers to infect themselves by eating raw Sarcosporidial meat, with no effect. Mice, on the other hand, have been infected by feeding them with meat containing *Sarcocystis tenella*, which is normally found in sheep, and also by feeding with mouse flesh normally infected with *Sarcocystus muris* (the mouse Sarcocyst) so that in the case of mice the infection is not always specific.

In herbivorous animals a number of difficulties occur in attempting to explain the transmission. It seems possible, however, that the spread may be caused through the animals accidentally eating with their food, the droppings of infected animals.

Ticks and biting insects of various sorts have been blamed for spreading Sarcosporidiosis.

SARCOCYSTIN—THE TOXIN SECRETED BY THE SARCOCYST.

The Sarcosporidia are the only Protozoa from which a definite toxin has been extracted, and this toxin acts in many ways similarly to that of Bacteria.

Teichmann and Braun (22) state that the discovery of a toxin produced by *Sarcosporidia* was made by Pfeffer in the year 1889.

Teichmann (21) working on the Sarcosporidia of sheep, discovered that they contain a poison which is particularly toxic to rabbits. He found that the lethal dose for rabbits was 0.0002 gm. of the dried Sarcocyst. The action appears to be on the central nervous system and to affect in some way the contained lipoids. The toxin appears to be destroyed by heating to 100 degrees centigrade.

Teichmann (21) also found that this toxin could produce both active and passive immunity in rabbits, but that the toxin could not be separated from the antitoxin in a neutral mixture.

Teichmann and Braun (22) give the following list of properties of Sarcocystin:—

(a) It is thermolabile;
(b) Filterable;
(c) Soluble in physiological salt solution;
(d) It is only toxic for rabbits, not for other animals. The natural immunity of these has nothing to do with the antitoxic capacity of their serum.

These two authors show that Sarcocystin is composed of two entities, one toxic in action which is destroyed by heating to 60°C., and the other which causes hæmolysis in the blood of sheep, guinea pigs, horses, and doves, but not rabbits, is not destroyed at this temperature.

The authors also state that immune serum contains complementary antibodies against the Sarcosporidian extract.

SYSTEMATIC POSITION OF THE SARCOSPORIDIA.

The group is placed by most authors amongst the *Neosporidia*. Castellani and Chambers (3) place them as the fourth order of the Neosporidia, as do Braun and Lühe (2). Kisskalt and Hartmann place them provisionally among the *Cnidosporidia*.

Both Minchin (18) and Hartog, in the Cambridge Natural History (11), and Daniels and Newham (4) stating that it is the continuous formation of spores, without the destruction of the formative cells, which separates the *Sarcosporidia* from the associated forms of the *Myxosporidia* which occur in fish.

Wenyon (23) considers the *Sarcosporidia* to be parasites of undetermined position, and even suggests that they may be fungi.

The classification given by Hartog in the Cambridge Natural History (11) is as follows:—

PHYLUM, PROTOZOA.
CLASS, SPOROZOA.
SECTION B, NEOSPORIDIA.
ORDER 3, SARCOSPORIDIACIAE.

"Encysted parasites in the muscles of Vertebrates, with a double membrane, spores simple."

RECORDED NUMBER OF SPECIES.

Wenyon (24) records thirty-nine species of Sarcocystis up to 1926. Of these twenty-eight have been found in Mammals, of which one species, *Sarcocystis darlingi* (Brumpt, 1913), has been obtained from a Marsupial, an opossum. Nine have been found in Birds, and two in Lizards.

AUSTRALIAN OCCURRENCE.

Sarcocystis mieschleriana (Kuhn) has been found in pigs in Western Australia and other States of the Commonwealth. *Balbiana gigantea* (=*Sarcocystis tenella*), which causes œsophageal tumours, has been found according to Johnson and Cleland (13), in sheep in New South Wales and Western Australia. The authors consider that the latter sheep were probably imported from Victoria. *Sarcocystis tenella* is common in the skeletal muscles of sheep in Western Australia.

Johnson and Cleland (13) record the following *Sarcosporidia*:—

Sarcocystis miescheriana (Kuhn), Muscles of Western Australian pigs.

Balbiana gigantea (*Sarcocystis tenella* (Raill)), in New South Wales and Western Australian sheep.

Sarcocystis blanchardi (Dofl), in cattle in New South Wales; affecting tongue and heart muscles.

Sarcocystis muris (Blanch): Recorded from Sydney in *Mus rattus* and *Mus decumanus*.

Gilruth (10) records *Sarcocystis* Sp. in the muscles of sheep and cattle in New South Wales and in Victorian sheep, infecting the tongue. He also records *Balbiana gigantea* from New Zealand sheep.

Gilruth and Bull (9) record *Sarcocystis macropodis* from *Petrogale*, and *Ileocystis* and *Lymphocystis* from *Macropus* Sp.

There appears to be some confusion concerning the presence of Sarcosporidia in Marsupials. A number of European authors state that Sarcosporidia have been found in the Kangaroo, but Wenyon's only record up to 1926 in Marsupials is that of their occurrence in an Opossum, and the specimen *Sarcocystis macropodis* recorded by Gilruth and Bull from the Rock Wallaby *Petrogale* was in the form of cysts in the submucosa of the stomach and did not occupy the more usual position in the actual muscle fibres. Searches through the Australian Science abstracts from 1922 to 1932, the Zoological records from 1864 to 1932, and the Biological abstracts from 1926 to 1932 have not revealed any further reference to Sarcosporidia in Marsupials, so, presumably, the origin of the statement comes from the record of *Sarcocystis macropodis* from the *Petrogale.*

Tiegs (23) records a new species, *Sarcocystis pythonis* from the Australian Carpet Snake (*Python spolotes*).

PROBABLE NEW OCCURRENCE IN WESTERN AUSTRALIA.

One of a number of white mice which were supplied to the author by Dr. H. W. Bennetts, D.V.Sc., for experimental X-ray work, showed the presence in the thigh muscles of definite Sarcosporidia, which were considered, for the following reasons, to be the Mouse Sarcocyst:—

Sarcocystis muris:

(1) The mouse assumed a very peculiar gait, eventually became puffed up and died. This extreme pathogenicity is characteristic of *Sarcoystis muris* in mice.

(2) The parasties were present in the thigh muscles, the usual site of infection for this species of Sarcocyst.

(3) The parasites had grown along the muscle fibre to a relatively enormous length, that is 2 to 4 cms.

(4) The spores measured $14\mu \times 2{\cdot}7\mu$, which is within the limits of variation for this species of Sarcosporidia.

Sarcocystis muris has not previously been found in Western Australia, and although this is the first record of its presence here, it is not yet definitely known to be endemic, since the mouse in which it has been found was a descendant of stock originally sent from South Australia. The parasite is therefore probably imported.

RECORD OF A NEW SPECIES OF SARCOSPORIDIA FOR WESTERN AUSTRALIA.

In sections of muscle from the back of a Western Australian Marsupial (*Bettongia grayi*) a Sarcosporidian was found occupying the muscle fibres, which has been regarded as a new species.

This is the first record of one of these parasites in the Western Australina Marsupial fauna, and the first record in the skeletal muscles of an Australian Marsupial.

The infection was localised, but heavy. It occurred on the back muscles to the left of the vertebral column, but not on the right, just cranial to the region where the tail sinews join the back muscles.

Unfortunately the parasites were not known to be present until the sections had been made and mounted, so that there was no tissue available in order to make a detailed examination of the spores.

From an examination of the sections, the spores appeared to be sickle-shaped, that is, they were typically Sarcosporidian in form. Some of the parasites reached up to a centimetre in length and appeared to be made up throughout their length of a mass of spores. There is very little data on which to erect a new species, but as the majority of Sarcocysts appeared to have been classified according to the host infected, the name *Sarcocystis bettongiæ* is therefore suggested for this new Sarcosporidian from Western Australia.

Sarcocystis bettongiæ (Bourne, 1932), *Host. Bettongia lesueuri grayi* (Gould).

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PLATE I.

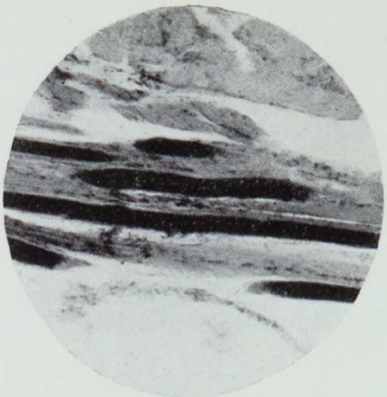

Fig. 1

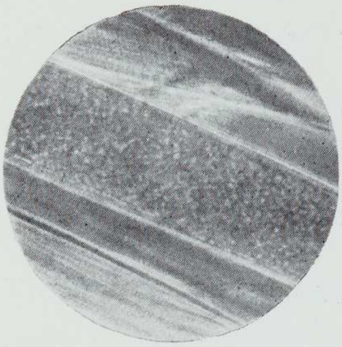

Fig. 4

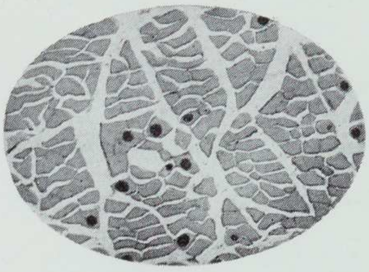

Fig. 5

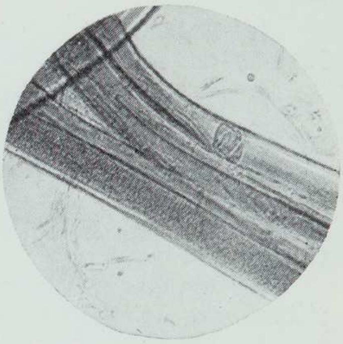

Fig. 3

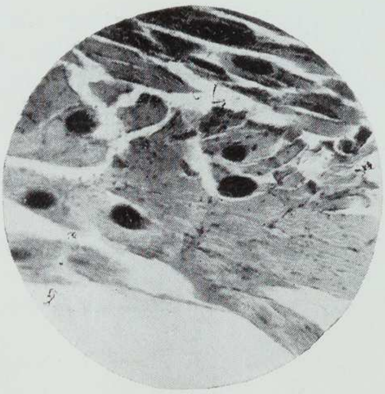

Fig. 2

Magnification: Fig. 1, 2, and 3, × 300; Fig. 4, × 600; Fig. 5, × 200.

Explanation of Plate.

Fig. 1.—Longitudinal section of muscle of *Bettongia grayi,* showing **Sarcocystis bettongiae** Sp. n. in fibres.

Fig. 2.—Transverse section of muscle of *Bettongia grayi,* showing **Sarcocystis bettongiae** Sp. n. in fibres.

Fig. 3.—Teased muscle fibres of white mouse, showing *Sarcoystis muris* in lower fibre.

Fig. 4.—Enlarged portion of Fig. 3, showing the Sarcocyst occupying practically the entire fibre.

Fig. 5.—Transverse section of muscle of white mouse showing *Sarcocystis muris* in muscle fibres.

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